White Biotechnology for added value products from renewable plant polymers: Design of tailor-made biocatalysts and new Industrial bioprocesses

BIORENEW

SWP4.1 : "Functionalisation of cellulosic fibres“

Characterization of laccase oxidation of lignosulfonates in presence of lignocellulosic substrates

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Abstract

Laccases (EC 1.10.3.2) are multi–copper oxidases, which catalyze one electron oxidation of a wide range of inorganic and organic substances, coupled with one four-electron reduction of oxygen to water (Xu 1996). Laccases not only catalyze the removal of a hydrogen atom from the hydroxyl group of methoxy-substituted monophenols, *ortho*- and *para*-diphenols, but also can oxidize other substrates such as aromatic amines, syringaldazine, and non-phenolic compounds, to form free radicals (Bourbonnais *et al.* 1997, Li *et al.* 1999, Robles *et al.* 2000; Durán and Esposito 2000). It is known that laccases can catalyze the polymerization of various phenols and halogen, alkyl- and alkoxy-substituted anilines (Hoff *et al.* 1985, Kobayashi *et al.* 2001, Kobayashi and Higashimura 2003). Only recently has positively been demonstrated that plant laccases are able to polymerize monolignols within the plant cell wall matrix, in the complete absence of peroxidase (Sterjiades *et al.* 1992, Liu *et al.*1994, Richardson *et al.* 2000) and to break down non-phenolic ligno-cellulose by certain phenolic compounds acting as mediators (Bourbonnais *et al.* 1997). These studies show that laccases are involved only in the early stages of lignification, while peroxidases are involved later (Bao *et al.* 1993, Wallace & Fry 1999, Boudet 2000). Thus, the oxidation of lignosulfonates with laccase to binding fiber, particle and paper boards could be an important achievement to overcome the environmental limitation of the phenol–formaldehyde resins. The objective of this preliminary work is to characterize four different lignosulfonates after laccase oxidation

in order to understand their potentiality toward industrial application as cross-linker for paper and lignocellulosic substrates.

The characterization of the four lignosulfonates has shown similar results in term of color and chemical structure as confirmed by the Uv-Vis and FT-IR analysis. However, the particle size of the DP399 showed higher values then DP398 (6.7 and 9.5 nm respectively) indicating that slight changes in the chemical structure can have visible changes in the organization behavior of the lignosulfonates in solution. The lignosulfonate DP400 and DP401 have shown different chemical structure both in Uv-vis and FT-IR spectra and a different particle size range (7.1 and 5.1 nm).

As the highest concentration of enzyme was the responsible for the maximum change in the absorbance spectrum the 20 U/mL was the activity selected to perform the assays. The laccase oxidation of the four lignosulfonate showed dramatic changes in the chemical structure of all the samples that could be attributed to the conversion of the oxidized phenolic hydroxyl groups. For all the samples the UV peaks at 250 and 280 nm disappeared and the peaks at 320 and 360 nm are significantly decreased and the visible absorbance showed a general increase in all spectra. In the visible spectra the laccase oxidized lignosulfonates DP398 and DP399 showed similar behavior with the formation of a large shoulder around 400-450 nm. However, the lignosulfonates DP400 and DP401 showed different behavior with the formation of a narrow peak at 650 nm for DP400 and at 450 nm for DP401. The comparison of the FT-IR spectra of the oxidized and raw lignosulfonates showed a similar behavior in all the samples. The growing in the band of the typical aromatic skeletal vibration bands (1600 and 1510 cm−1) and the decreasing in the bands of the ether group and of the C-O, C-H and C=O vibration (between 1300 and 1000 cm−1) indicate a breakdowns of the lignosulfonate structure and a reassembling of the aromatic skeletal in a different polymer structure. The particle size results confirmed this conclusion indicating a growing in the oxidized lignosulfonates size, in special for the DP401 (102.6%), and a decreasing in the polydispersity, in special for DP400 (48.1%), that indicate the decrease of the heterogeneity of the compounds. The K/S values of the lignocellulosic fibers have shown that the oxidized lignosulfonate compounds catalyzed by laccase have poor affinity toward fibers. However, the oxidized lignosulfonate showed significant differences in the UV-vis spectra. The differences between the flax and sisal could be attributed to the different percentage of lignin in the two substrates. In conclusion, the results showed that a huge oxidation of the aromatic structure of the lignosulfonates was achieved and a reorganization of the oxidized lignosulfonate by crosslinking reactions leads to the formation of a new polymer structure.